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Improving fenugreek plants growth and productivity via humic acid treatment

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ABSTRACT

Fenugreek is a commonly consumed herb due to its medicinal properties and nutritional value. Humic acid (HA) at 0, 50, 100, 200, 300 and 400 ppm were applied as a seed priming, soil addition, foliar spray, seed priming + soil addition and soil addition + foliar spray. Both of HA concentrations and method of applications significantly improved plant growth and productivity. Moreover, there was a significant interaction between the two investigated factors. Plant height, plant dry weight, number of pods/plant and seed yield were generally increased by increasing HA concentrations regardless the method of applications. Soil addition had the highest significant values for all investigated traits regardless the concentration of HA. Overall, plants which treated with 400 ppm HA as soil addition + foliar spray had the highest significant number of pods/plant (257.77) and seed yield (68.09 g/plant). Whereas, HA-untreated plants had the lowest values (71.27 pod/plant and 21.33 g/plant). Overall plants treated with 400 ppm HA as a soil addition and foliar spray had the highest yield 2587 kg/fed. however, control plants which developed from priming seeds had the lowest yield 746 kg/fed. Therefore, it is recommended to treated fenugreek plant with HA as a soil addition and foliar spray three times during the growth season to increase seed yield by about 3-fold compared with untreated plants.

KEYWORDS:

1. INTRODUCTION

Fenugreek plant (*Trigonella foenum-graecum* L.), is an annual herb of Fabaceae family. It is native to the Mediterranean area and widely grown in many countries (De Candolle, 1964). Stems, leaves, seeds and twigs of the plant are used for cooking and medicinal properties. Both of seeds and leaves of fenugreek plants have a high medicinal and nutritional value. The seeds are a rich source of carbohydrates, lipids, proteins, mucilaginous fibre, vitamins A and C, calcium, iron, and other nutritional minerals (Sauvaire *et al*., 1976 ; Billaud and Adrian, 2001). Dahanukar *et al.* (2000) added that they are also, rich in many secondary metabolites, such as trigonelline, coumarin, saponins, nicotinic acid, sapogenins, and phytic acid. Fenugreek is consumed to prevent constipation, improve digestion, stimulate the liver and spleen, increase the erythrocyte insulin receptors, enhance pancreatic function, purify the blood and improve appetite (Ahmadiani *et al.*, 2001 ; Randhir *et al.*, 2004).

Humic acid (HA) has been known since long time as vital contributors to soil fertility. Acting as a soil physicochemical and biological amendment it improves crop growth and productivity (Canellas *et al*., 2015). Humic acid is an outstanding natural and organic way to provide plants and soil with a concentrated dose of nutrients, vitamins and trace elements. Furthermore, it improves the uptake of micro- and macronutrients, by enhancing soil cation exchange capacity, and P availability via interfering with calcium phosphate precipitation (Trevisan *et al*., 2010; Jindo *et al*., 2012).

Different HA products are widely offered commercially for soil and foliar application. Rose *et al*. (2014) reviewed the response of different crops to HA application and concluded that the biomass of many crops was increased, but the responses were highly variable depending on the type of crop and environmental conditions. Halpern *et al*. (2015) and Canellas *et al*. (2015) reviewed agricultural application of HA, citing many examples where yield and quality of different horticultural crops were improved. They elucidated complex interactions between plants and HA which finally stimulated their growth with variation based on doses and times of applications. Lyons and Genc (2016) and Olk *et al*. (2018) showed favorable plant responses to commercial HA products but they emphasized inconsistency, mentioning that they

often improve crop yield through alleviation of different environmental stresses.

On mungbean plants Waqas *et al*. (2014) emphasis that humic acid as seed priming, foliar spray or soil addition significantly improved number of pods/plant, seeds/pod and seed yield. However, the biological yield was not significantly affected by method of applications. Soil addition of HA 1, 2 or 3 kg/ha resulted in higher number of pods/plant and grain yield, with no statistically difference among these concentrations. Dawood *et al*. (2019) showed that foliar spray of faba bean plants with 5 ml/l HA significantly improved vegetative growth traits, yield, and some chemical constitutes of seeds. Humic acid as a foliar application increased soybean yield but never influenced seed oil concentration. However, seed protein content was reduced depending on the environmental conditions (Lenssen *et al*., 2019). Kahraman (2017) treated chickpea seeds with HA at 0, 60, 90 and 120 kg/h divide into 2 doses (pre-sowing and pre-flowering) and found a positive effect of the treatments depending on the concentration.

Therefore, this study was conducted to assess the effects of different concentrations of HA and method of application: seed priming, soil addition, and foliar application on growth and productivity of fenugreek plants**.**

2. MATERIAL AND METHODS

This investigation was carried out during the two consecutive seasons 2017/2018 and 2018/2019 at the nursery and laboratory of ornamental plants, Fac. of Agric., Minia Univ. The physical and chemical analysis of the experimental soil described by Black *et al.* (1981) is shown in table 1.

Table 1. Physical and chemical analysis of the experimental soil

Soil characters	Value	Soil characters		Value
Soil type	Clay loam	Avail. P (%)		15.40
Sand (%)	28.59	Exch. K (mg/100g)		2.45
Silt (%)	30.29	Exch. Ca (mg/100g)		31.43
Clay	41.12	Exch. Na (mg/100g)		2.46
Organic Matter (%)	1.65	DTPA Ext.(ppm)	Fe	8.39
$CaCo_3$ (%)	2.10		Cu	2.04
PH (1:2.5)	7.79		Zn	2.81
EC (mmhos/cm)	1.06		Mn	8.19
Total N (%)	0.08			

A complete randomized block design with 3 replicates in a split plot arrangement was conducted. The main plot was 3.6×4.2 m with 60 cm distance between the rows and 40 cm between the hills within the row. The main plot included 5 methods of HA application which were seed priming, soil addition, foliar spray, seed priming + foliar spray and soil addition + foliar spray. The subplot included 6 concentrations of HA (0, 50, 100, 200, 300, and 400 ppm). Therefore, the experiment included 30 treatments.

Seeds were primed for 24h before sowing on HA concentrations using 2 liters for each 1kg of seeds. Treated seeds were sown on 26th Oct. in both seasons and plants were thinned twice, finally each hill contained 2 plants. Plants received thrice foliar sprays of HA concentrations with 2 weeks intervals, till run off starting on 23rd Nov. For soil application 50 ml of HA was added around the base of the plant three times on the same time of foliar treatment.

All plants received NPK fertilizers at 50 kg/fed. of ammonium sulphate (20.6% N), 200 kg/fed. of calcium superphosphate (15.5% P_2O_5) and 50 kg/fed. of potassium sulphate (48% K_2O). Phosphorus fertilizer was added during preparing of the soil to cultivation, while K fertilizer was added on 26th November in both growing seasons. The N fertilizer was divided into two batches, added with 3 weeks intervals, starting on 26th Nov. All other agriculture practices were carried out following farmer habitat.

At maturity stage (25th March) plants were cut just above ground. Plant height was measured from the ground to the top most point of the plant. After harvesting, the plants were dried for several days to remove moisture from them. Individual plants were weighted to record dry weights after air-drying. Pods number and seed yield were assessed after their extracting manually from each plant and then weighted. The obtained data were tabulated and subjected to proper statistical and analysis according to Mead *et al.* (1993) using the statistical program MSTAT-C.

3. RESULTS AND DISCUSSION

3.1. Plant height

The analysis of variance showed that both of HA concentrations and method of application affected the height of fenugreek plant ($p \leq 0.05$). Moreover, there was a significant interaction between these two factors as shown in table 2, with a

Table 2. Effect of concentrations and method of applications of humic acid on the plant height of fenugreek plant during two seasons.

Humic acid concentrations (ppm) (B)	Method of applications (A)					Mean (B)
	Seed Priming (SP)	Soil Addition (SA)	Foliar Spray (FS)	SP + FS	SA +FS	
First season (2017/2018)						
0	63.02	62.51	64.00	63.55	64.01	62.50
50	69.61	70.44	72.07	76.91	79.31	73.67
100	73.53	76.18	77.90	84.54	87.02	79.83
200	78.21	82.72	87.89	95.05	95.29	87.83
300	82.76	89.49	95.62	103.31	104.92	95.22
400	88.10	95.12	106.22	110.60	115.30	103.07
Mean (A)	75.09	79.41	84.01	88.99	90.65	
L.S.D. at 5%		A: 1.21	B: 1.72	AB: 3.84		
Second season (2018/2019)						
0	62.56	61.53	63.51	61.93	64.22	62.7
50	68.84	69.87	72.69	76.55	79.43	73.5
100	74.92	75.89	77.88	84.3	86.62	79.9
200	80.92	83.34	87.42	94.85	95.49	88.4
300	86.86	90.29	94.76	103.83	106.93	96.5
400	93.51	99.19	107.69	106.93	116.93	104.8
Mean (A)	77.94	80.02	83.99	96.53	105.72	
L.S.D. at 5%		A: 1.57	B: 1.23	AB: 2.74		

similar trend in both seasons. In the 1st one, plant height was varied between 75.09 and 90.65 cm depending on the method of application. Humic acid-untreated plants had the shortest plant height (62.50 cm) which gradually increased to the maximum value (103.07 cm) once plants treated with the highest concentration of HA (400 ppm). Overall, there was no significant difference among method of application for plants treated with tap water. Almost any increase on HA concentrations was coincided with significant increase on plant height under any method of applications. The highest plant height (115.30 cm) being for plants treated with 400 ppm of HA as a soil addition and foliar spray whereas, the shortest plants (62.5 cm) were those untreated with HA (Table 2).

3.2. Plant dry weight

The ANOVA showed a significant variation on fenugreek plants dry weights due to concentrations of HA as well as, method of applications. Moreover, there was a significant interaction between both factors. Generally, there was an increment on the plant dry weights by increasing concentrations of HA in both seasons. Overall, soil addition + foliar spray had the superior effect than the other method of applications under the same concentration of HA. In the 1st season, plants developed from HA-untreated seeds under different method of applications had the lowest value (19.65 g) whereas, the highest one (39.08 g) was obtained when seeds were treated with 400 ppm of HA as a soil addition + foliar spray. Regardless the method of applications there was a liner increase in plant dry weights by increasing concentrations of HA (Table 3). Similar results were achieved in the 2nd season.

3.3. Pod number

Table 4 shows similar response trend for fenugreek plants to HA application in both experimental seasons. Concentrations of HA as well as method of applications significantly affected number of pods/plant which gradually increased by increasing the concentrations of HA. Also, there was a significant difference among the methods of applications. For example in the 1st season increasing HA concentrations from zero to 400 ppm increased number of pods from 71.37 to 163.13 pods/plant. Also, numbers of pods increased from 112.88/plant for seed priming treatment to 166.30 for plants received HA as a soil addition + foliar spray. Results showed a significant interaction between the two investigated factors. Regardless the method of applications almost any increase on HA concentrations increased the number of pods

Table 3. Effect of concentrations and method of applications of humic acid on the dry weights (g/plant) of fenugreek plant during two seasons.

Humic acid concentrations (ppm) (B)	Method of applications (A)					Mean (B)
	Seed Priming (SP)	Soil Addition (SA)	Foliar Spray (FS)	SP + FS	SA +FS	
First season (2017/2018)						
0	18.30	19.12	19.99	20.87	19.95	19.65
50	21.11	23.00	23.59	23.80	25.02	23.30
100	23.00	24.37	25.79	25.84	26.82	25.16
200	25.25	26.95	27.97	29.04	29.92	27.83
300	26.46	29.39	31.04	33.12	34.56	30.91
400	29.10	34.91	32.79	37.07	39.08	34.59
Mean (A)	23.87	26.29	26.86	28.29	29.23	
L.S.D. at 5%		A: 1.77	B: 0.87	AB: 1.94		
Second season (2018/2019)						
0	19.24	20.24	20.56	20.44	21.14	20.32
50	21.17	22.71	23.55	24.44	24.57	23.29
100	22.93	24.86	25.64	26.97	27.89	25.66
200	25.72	28.17	30.34	31.51	33.95	29.94
300	27.85	30.80	35.58	36.40	38.63	33.85
400	29.65	34.24	39.25	41.78	44.69	37.92
Mean (A)	24.43	26.84	29.15	30.26	31.81	
L.S.D. at 5%		A: 1.91	B: 1.12	AB: 2.50		

Table 4. Effect of concentrations and method of applications of humic acid on the pod number of fenugreek plant during two seasons.

Humic acid concentrations (ppm) (B)	Method of applications (A)					Mean (B)
	Seed Priming (SP)	Soil Addition (SA)	Foliar Spray (FS)	SP + FS	SA +FS	
First season (2017/2018)						
0	89.68	88.52	89.09	88.62	90.54	71.37
50	96.12	103.48	114.52	121.11	120.63	91.95
100	107.96	112.50	129.40	137.85	145.30	105.01
200	117.34	126.32	153.50	167.4	182.00	125.84
300	127.56	140.48	172.08	192.16	221.58	145.26
400	138.63	150.51	190.28	217.07	257.77	163.13
Mean (A)	112.88	120.30	141.48	153.93	166.30	
L.S.D. at 5%		A: 1.16	B: 1.90	AB 2.03		
Second season (2018/2019)						
Control	91.02	90.5	89.37	90.14	91.72	90.75
50	97.15	107.01	116.38	123.86	122.79	113.44
100	109.96	115.35	130.47	141.01	147.96	128.95
200	120.53	128.63	154.86	169.4	186.05	151.89
300	129.73	142.2	174.02	190.42	223.66	172.01
400	140.78	154.33	190.29	223.41	265.89	194.94
Mean (A)	114.86	123.00	142.57	156.37	172.09	
L.S.D. at 5%		A: 1.08	B: 1.06	AB 2.36		

(P≤0.5). The minimum number of pods was for HA-untreated plants with no significant difference due to method of applications. The highest number of pods (257.77 /plant) being for plants treated with 400 ppm of HA as a soil addition and foliar spray. Similar results were observed in the 2nd season (Table 4)

3.4. Seed weight/plant

Both of investigated factors increased (p≤0.05) the yield of fenugreek seed/plant with a similar trend in both seasons. There was a significant interaction among these two factors. In the 1st season, HA-untreated plants under different method of applications had similar yield (19.63 -22.50 g) with no significant difference among them. There was no significant effect of 50 ppm HA on seed yield when it applied as a seed priming or soil addition whereas, other method of applications significantly increased seed yield. Under any method of applications seed yield significantly increased by the gradual increase of HA concentrations from 100 to 400 ppm. The highest yield in the two seasons (68.09 and 71.08 g, respectively) was estimated for plants treated with 400 ppm HA as a soil addition + foliar spray. The second promising treatment was the same concentration of HA but applies as seed priming + foliar spray which yielded 62.86 g/plant (Table 5).

Table 5. Effect of concentrations and method of applications of humic acid on the seed yield (g/plant) of fenugreek plant during two seasons.

Humic acid concentrations (ppm) (B)	Method of applications (A)					Mean (B)
	Seed Priming (SP)	Soil Addition (SA)	Foliar Spray (FS)	SP + FS	SA +FS	
First season (2017/2018)						
Control	19.63	21.47	22.50	20.30	21.76	21.23
50	21.81	23.89	25.28	26.02	30.13	25.43
100	24.24	26.40	31.31	29.20	35.42	29.31
200	28.79	29.67	37.59	38.47	43.38	35.58
300	34.87	38.95	42.73	48.97	52.53	43.61
400	40.23	47.73	57.19	62.86	68.09	55.26
Mean (A)	28.26	31.38	36.10	37.64	41.05	
L.S.D. at 5%		A: 0.71	B: 1.05	AB 2.35		
Second season (2018/2019)						
Control	20.39	21.33	21.74	22.15	22.51	21.62
50	22.12	24.26	26.82	25.98	26.20	25.08
100	23.51	27.12	31.34	34.51	34.84	30.26
200	29.24	33.31	37.76	43.09	44.23	37.53
300	34.47	43.78	45.02	53.50	54.94	46.34
400	39.86	51.55	57.51	63.84	71.08	56.77
Mean (A)	28.27	33.56	36.70	40.51	42.30	
L.S.D. at 5%		A: 0.86	B: 0.92	AB 2.05		

3.5. Seed yield/fed.

Seed yield/fad had the same trend as the seed yield/plant (Table 6). The analysis of variance showed a significant effect of both factors with a significant interaction between them. Overall plants treated with 400 ppm HA as a soil addition + foliar spray had the highest yield 2587 and 2708 kg/fad in both seasons, respectively. However, control plants which developed from priming seeds had the lowest yield 746 and 777 kg/fad in both season, respectively, with no significant difference among control plants due to method of application.

Our results showed that the effect of HA on fenugreek plants was linearly correlated with its concentrations up to 400 ppm which had the highest significant increase in plant heights and dry weights (63 and 77%, respectively) over that of untreated plants in the 1st season. This improvement in plant growth resulted in augmentation on number of pods and seed yield/plant which increased by 84 and 159%, respectively with similar observation in the 2nd season. The promotion effect of HA on many legume crops has been widely reported (Ashraf *et al*., 2005, Waqas *et al*., 2014; Karaman, 2017; Li *et al*., 2019).

Table 6. Effect of concentrations and method of applications of humic acid on the seed yield (kg/fed.) of fenugreek plant during two seasons.

Humic acid concentrations (ppm) (B)	Method of applications (A)					Mean (B)
	Seed Priming (SP)	Soil Addition (SA)	Foliar Spray (FS)	SP + FS	SA +FS	
First season (2017/2018)						
Control	746	816	855	771	827	807
50	829	908	961	989	1145	966
100	921	1003	1190	1110	1346	1114
200	1094	1127	1428	1462	1648	1352
300	1325	1480	1624	1861	1996	1657
400	1529	1814	2173	2389	2587	2100
Mean (A)	1074	1192	1372	1430	1592	
L.S.D. at 5%		A: 27	B: 37	AB 83		
Second season (2018/2019)						
Control	777	812	828	844	858	824
50	843	924	1022	989	998	955
100	896	1033	1194	1314	1327	1153
200	1114	1269	1438	1641	1685	1429
300	1313	1667	1715	2038	2063	1759
400	1549	1964	2191	2432	2708	2169
Mean (A)	1082	1278	1398	1543	1607	1381
L.S.D. at 5%		A:39.61	B:35.78	AB:80.01		

Besides enhancement nutrients uptake with HA application Rose *et al*. (2014) and Vista (2017) suggested that it also, contributes to cell wall loosening, cell enlargement leading to organ growth. Another explanation for increasing plant height and biomass might that HA act in mode like plant growth substances (O'Donnel, 1973; Casenave de Sanfilippo *et al*., 1990)

Obtained data showed that methods of HA application had a significant effect on all estimated traits. Seed priming had the lowest effect followed by the soil addition. Yet, the dual treatment (soil addition + foliar spay) was the superior one as it increased number of pods and seed yield/plant by 50 and 50%, respectively, over that of seed priming treatment, in the 1st season. The superiority of different application methods over the seed priming one could be due to that plants developed form primed seeds had the minimum dose of HA compared with soil addition as plants on the last treatment which received three doses. Similarly foliar spray treatments provided plants with higher quantity of HA. Moreover, when applying HA as a foliar treatment some of it also reaches to the soil that could make a synergetic effect during uptake of nutrients (David *et al*., 1994). For the same reason, results could explain why the dual treatments were superior to single one. Kaya *et al*. (2005) suggested existence of synergetic effect of mutual applications of HA and mineral nutrients.

Recent results are in line with those found by Kahraman (2017) and Ashraf *et al*. (2005) as soil application yielded significantly higher yield than foliar spray which was better than seed priming one. However, in all cases the response depended on the dose of application. Nonetheless, our results do not agree with those of Waqas *et al*. (2014) on mungbean as method of application did not affect the biological yield. Foliar uptake of nutrients is widely accompaniments standard root fertilizer application (Peigne *et al*., 2018) and recently Smilkova *et al*. (2019) confirmed the uptake of humic substance via cuticular diffusion.

The current study revealed a significant interaction between HA concentrations and methods of applications for all investigated traits. Overall, number of pods and seed yield/plant for plants treated with 400 ppm HA as a soil addition + foliar spray were increased in the 2nd season by 187 and 240% respectively over that of the untreated plants with similar improvement in the 1st season. Overall, in that season plants treated with 400 ppm HA as a soil addition + foliar spray had the highest yield 2587 kg/fed. However, control plants which developed from priming seeds had the lowest yield

746 with no significant difference among control plants due to method of application

Li *et al*. (2019) explained the mechanism of improving of peanut crop following HA fertilization by evaluating various soil characters and concluded that it amplified soil N, P and K contents and availability. Likewise, the stimulating response of HA has been partially related to enhance uptake of macro and microelements (Calov *et al*., 2014 ; Canellas *et al*., 2014; Yakhin *et al*., 2017; Halpern *et al*., 2017).

As a conclusion this study suggested that treated fenugreek plants with humic acid at 400 ppm as a soil addition + foliar application three times during the growth season could significantly improve the plant growth. This treatment increased seed yield more than 3-fold than plants developed from HA-priming seed.

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الملخص العربى

تحسين نمو وانتاجية نباتات الحلبة باستعمال حامض الهيوميك

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الحلبة هي نبات عشبى شائع الاستهلاك لخصائصه الطبية وقيمته الغذائية. تم معاملة النبات بحامض الهيوميك بتركيز ٠ ، ٥٠، ١٠٠، ٢٠٠، ٣٠٠ و ٤٠٠ جزء في المليون وذلك بعدة طرق وهى اما نقعاً للبذور قبل الزراعة، إضافة التربة، الرش الورقي، نقع البذور + إضافة التربة، إضافة التربة + الرش الورقي. أدت كل من تركيزات الحامض وطريقة التطبيقات إلى تحسين نمو النبات وإنتاجيته بشكل كبير. علاوة على ذلك، كان هناك تفاعل كبير بين العاملين قيد الدراسة. تم زيادة ارتفاع النبات والوزن الجاف للنبات وعدد القرون/نبات ومحصول البذور بشكل عام عن طريق زيادة تركيزات حامض الهيوميك بغض النظر عن طريقة الاستخدام. كان لإضافة التربة أعلى القيم المعنوية لجميع الصفات التي تم دراستها بغض النظر عن التركيز. بشكل عام، كان للنباتات التي تم معاملتها بـ ٤٠٠ جزء في المليون من الحامض كإضافة للتربة + رش على الأوراق أكبر عدد من القرون/نبات (٢٥٧.٧٧) ومحصول البذور (٦٨.٠٩ جم /نبات). في حين أن النباتات غير المعاملة كانت لها أدنى قيم (٧١.٢٧ قرن/نبات و ٢١.٣٣ جم /نبات). وكان للنباتات المعاملة بـ ٤٠٠ جزء في المليون من الحامض كإضافة للتربة + الرش الورقي أعلى محصول للفدان ٢٥٨٧ كجم. أما النباتات التي نمت من بذور غير معاملة كانت أقل إنتاجية حيث بلغت ٧٤٦ كجم/فدان. لذلك يوصى بمعاملة نبات الحلبة بحمض الهيوميك كإضافة للتربة + الرش الورقي ثلاث مرات خلال موسم النمو لزيادة محصول البذور بنحو ٣ أضعاف مقارنة بالنباتات غير المعاملة.